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By

Annah Putnam Hazen.

With 11 figures in text.

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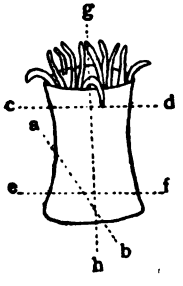
While working on the regeneration of the oesophagus in small pieces of the anemone, Sagartia luciae, it was noticed that the attachment of the pieces to the bottom of the dish occurred, in nearly all cases, before the tentacles appeared. The pieces had been placed in the dishes without regard to orientation. A later study of the sections seemed to show from the arrangement of the mesenteries that the orientation of the regenerating pieces was sometimes different from that of the individuals from which they had been cut, and it was suggested that contact might be one of the factors which determined the position of the new foot[1]). At that time no special attempt had been made to test this point, and, feeling that the suggestion was based on insufficient observation, I undertook some further experimental work, during the summer of 1902, at the Marine Biological Laboratory, Woods Hole, Mass., with the following problems in view: 1) to watch the regeneration of tentacles in aboral pieces, noticing particularly if tentacles developed with equal rapidity from pieces cut off at different levels, and if the absence of the old pedal disk made a difference in the time which elapsed between the operation and the appearance of tentacles; 2) to test the effect of gravity and contact on regenerating pieces; and 3) to determine whether the apparent change in orientation were permanent or not, and, if not, how the individual was able to right itself, especially in regard to its mesenteries.

[1]) The Regeneration of an Oesophagus in the Anemone, Sagartia luciae. Archiv f. Entwickelungsmech. XIV. 1902.

The regeneration of tentacles.

Experiment I. The first series of experiments was a repetition of those made a year ago, in which the pieces were cut as indicated by the line *a—b* Fig. 1 so that part of the old pedal disk was retained. The pieces were dropped into a dish of water without regard to orientation, and allowed to regenerate. The question arose, whether such pieces would succeed in placing themselves so that the old disk became attached to the dish, or whether the pieces would remain in the position which they took when first dropped into the water, and if so, whether a new disk would develop at the point of contact. The results which were obtained from a large number of individuals showed that the larger pieces oriented themselves at once, and became attached by the part of the original pedal disk within twenty-four hours. New tentacles regenerated on such pieces in three or four days. Many of the other pieces became attached after a somewhat longer time, and the formation of tentacles occurred in about the same time as above.

Fig. 1.



Certain orange stripes which are placed vertically on the surface of Sagartia luciae, parallel to the furrows, but not alternating with them, were plainly visible in the regenerating pieces, and showed the original orientation of the pieces. By means of these stripes, it was found that all these pieces had changed their position, whenever it had been necessary, and each one was fastened at the aboral end by its part of the old, pedal disk. The smallest pieces in this experiment, which did not become placed with their aboral ends down, remained unattached, and tentacles regenerated in a horizontal direction after a period of time which was always longer than was required for a similar development in the larger pieces which were attached. The presence of orange stripes on some of these small pieces showed that the horizontally lying tentacles were at one end — undoubtedly the oral, as shown by the later experiments. The original orientation had thus been maintained, although the piece was lying on its side. There was nothing observed to indicate that a new pedal disk developed at the place of contact, but, if these small pieces became attached at all, it was only often they had succeeded in turning the

aboral end down on to the bottom of the dish. In this, as in the other experiments, many of the smaller pieces diminished in size, and finally died.

Experiment II. From these facts it was evident that there was a certain amount of polarity in the pieces, and in order to ascertain how far this was influenced by the presence of the pedal disk, similar experiments were repeated on anemones from which the pedal disk had first been removed. (The two cuts were made as indicated by the lines *a—b* and *e—f* in Fig. 1.)

It is obviously difficult to make these cuts with desired accuracy as the anemones, when touched, contract rapidly into a small, round mass, and it is necessary to wait until they are again expanded before a second cut can be made. Even then, so much depends on the quickness with which the incision is made that the results are often not what is desired. The pieces cut in this way gave much the same result as those in Experiment I, with these exceptions, that the time required for pieces, which had no disk, to become fixed was longer, and a larger proportion of them regenerated tentacles without becoming attached than was shown by the pieces which retained a part of the old pedal disk. Thus showing that attachment was by no means necessary for the regeneration of tentacles.

Experiment III. To determine more exactly how much effect the presence of the foot might have upon the regeneration of tentacles, the foot was removed, as indicated by the line *e—f*, Fig. 1, and a second cut made in the plane of the line *c—d*. The pieces included between the two cuts were carefully watched. The average length of time for the appearance of tentacles was $4^5/_8$ days and the average time for the formation of an attachment disk was $6^4/_5$ days.

Experiment IV. For comparison with these results, the oral ends were cut from a number of anemones as indicated by the line *c—d*, Fig. 1, and the aboral pieces placed in water, the average time for the regeneration of tentacles was $3^8/_9$ days.

Experiment V. From other anemones, the oral ends were cut, leaving only a short aboral piece (see line *e—f*, Fig. 1) which regenerated tentacles after an average time of 5 days.

From these three experiments the results are not very conclusive, for, owing to the great extent to which the anemones expand, and change their shape, and the difficulty of making the cuts ac-

curate, it was impossible to compare the relative size of the different pieces with any degree of certainty. More work along this line did not seem profitable. We may, however, draw a few general conclusions. Tentacles regenerate more quickly on aboral pieces, cut at the line *c—d*, than at *e—f*, Fig. 1, but it is not shown whether this is due to a greater vitality of the larger pieces, or to a greater readiness to regenerate tentacles in the tissue from the oral region. In Experiments III and IV the surface upon which the tentacles regenerate is from about the same region, yet the time required for the development of tentacles is slightly longer in pieces without a pedal disk than in those which retain it. Experiment III shows that tentacles may regenerate when the foot is absent, and that the average length of time for the replacement of tentacles is shorter than required for the formation of a pedal disk. This indicates that the foot does not influence the regeneration of tentacles, and that the longer time required in Experiment III, when compared with experiment IV, is due to the smaller size of the pieces and the ill effects of so much cut-surface.

Experiment VI. Loeb found in Cerianthus membranaceus that tentacles and sometimes a mouth develops on the side of the body after a lateral incision is made. Since the individuals operated upon retained their complete ring of tentacles and mouth it would appear that the stimulation which produced the tentacles came from the exposed edge. I repeated this experiment on Sagartia luciae. In a number of cases a horizontal cut was made in the middle of the column, and in other individuals a vertical cut was made beginning behind the oral ring and extending some distance down the column. The pieces contracted and the cut edges closed immediately. All attempts to keep the incision open failed. After two days, a second incision was made in the same place, but the cut-edges closed again, and new tissue grew over the wound and for a time, a light unpigmented scar was present but no new tentacles grew on any of the specimens.

Experiment VII. A large number of anemones were cut in halves, longitudinally (shown by line *g—h*, Fig. 1). After the operation, the pieces closed making new individuals of one half the diameter of those which had been cut. The regeneration of tentacles on these pieces was watched in order to ascertain whether new tentacles grew out at the point where the cut edges joined, or whether they appeared between the old tentacles. This proved to be a diffi-

cult point to determine with accuracy, as sometimes there was no means of determining the exact position of the cut, and, moreover, it was often impossible to distinguish between the old tentacles, which were contracted, and the new ones which were developing. There was a short period, however, when the growing tentacles were so small that they could be positively identified, and the results from these cases, which seemed accurate, were recorded. Many of the specimens showed that the new growth was mainly in the region of the cut; a bunch of new tentacles appearing at that point. There were a number of interesting exceptions, however, of which Fig. 2, will serve as an example. This figure was drawn three days after the operation, and showed regenerating tentacles in two groups *a*

Fig. 2.

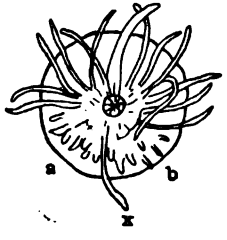
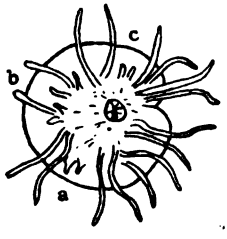


Fig. 3.



and *b* on one side of the oral ring with a single large tentacle *x* between the groups as if there had been a point of growth on both sides of this large tentacle. The other half of the oral ring, containing old tentacles, remained unchanged. Another exceptional case is represented by Fig. 3. Here the new tentacles were intercalated at three points *a*, *b* and *c* between the old tentacles. Other specimens varied slightly from these. While the evidence showed that there was a tendency for the tentacles to regenerate at the place of the cut, it was not very decided.

Experiment VIII. As it seemed possible that the position of the regenerating tentacles might correspond to that of the mesenteries which were being replaced, a group of tentacles were cut from one side of the oral ring leaving the mesenteries practically undisturbed. In a number of specimens the distal ends alone of tentacles were cut off but the short parts, which remained, so closely resembled the developing tentacles that it was difficult to distinguish them from new tentacles. In the majority of cases the developing tentacles came in on the cut surface but, in several instances a few new tentacles were

regenerated between the old ones, intercalated at various points around the oral disk. I hope later to be able to determine the position of regenerating mesenteries in specimens which have been divided longitudinally, and to discover whether they correspond to the developing tentacles or not.

Experiment IX. CERFONTAINE[1]) found that the oral piece of a polyp (Astroïdes calycularis), which had been divided transversely, regenerated a crown of tentacles, a mouth, and an oesophagus on the cut, aboral surface. I made a similar experiment with Sagartia, but while CERFONTAINE severed the polyps in the middle it was necessary to cut this anemone near the oral ring in order to get a heteromorphic regeneration. Otherwise a foot was formed. Some of the oral pieces were placed with the aboral end down, others with the oral end down. In a few instances tentacles began to develop, after four or five days, on the aboral ends of these pieces irrespective of their position in the dish, but the tentacles never grew out very far, and were absorbed in a short time.

Among the specimens which were placed with the aboral ends down a few slowly regenerated pedal disks. Some of the pieces which became fixed were very small, while larger ones occasionally seemed unable to become attached. It may be that in order to develop a foot, an oral piece must have a certain size relative to the individual from which it was taken. The contraction of the anemones made it impossible to demonstrate this point. The pieces which did not regenerate pedal disks gradually dimished in size, and died after a month or six weeks.

Effect of gravity and of contact.

Experiment X. The effect which gravity might have upon the time required for the regeneration of tentacles was tested in the following way: An aboral piece of an anemone was placed with the pedal disk touching a glass slide, and tied in position with a silk thread. Slides, fixed in this way, were placed in dishes of water, some vertically and others horizontally, the later with the oral ends down. They were thus arranged so that the oral-aboral axes of the anemones were either at right angles to gravity, or the oral end

[1]) Notes préliminaires sur l'organisation et le développement de différentes formes d'Authozoaires. Bull. de l'Acad. Roy. des Sci., des Lettres et des Beaux Arts de Belgique. No. 8. Notes V—VIII. 1891.

was down. Tentacles regenerated, in both cases, after three or four days showing by comparison with Experiment IV that tentacles develop downwards or in a horizontal direction as quickly as upwards. This might have been predicted since the anemones, when undisturbed are found everywhere, on the sides or bottoms of rocks, growing in the sand even, and indicating, as far as I have seen, that they have no decided preference in regard to orientation. The effect which contact might have upon orientation and regeneration would seem of more importance, since the anemones are fixed and the absence of a support, or the presence of a new point of contact might make considerable difference in the response of the individual. As was stated before, the pieces show a remarkable power to turn themselves over in order to become oriented in an upright position, the pieces might be cut and placed in the dishes with care, but on the following day it would be impossible to tell how many had changed their positions. To do away with these uncertain conditions, the following experiments were made.

Experiment XI. The aboral pieces of some anemones (cut as shown by the line *c*—*d*, Fig. 1) were placed on threads of cotton wool which had been pulled apart and were very loose, thus offering the slightest possible support without giving the pedal disk an opportunity to become attached or the piece to turn over. Other aboral ends were placed in the cotton wool, with the threads on the sides and top as well as below the pieces, making the contact nearly equal on all sides. Control experiments were made in which similar pieces were placed on the bottom of the dish. The results showed that tentacles developed as rapidly on the pieces in the cotton wool, as in those on the glass — the average time being a little over three days. Still other specimens, which were cut in the same way, were placed in the cotton wool some horizontally, and some vertically with the oral ends down. These pieces did not change their positions, but, in all cases regenerated tentacles at their oral ends after three or four days.

Experiment XII. Another way for practically removing contact from the specimens was found by suspending the aboral ends on a fine thread which had been drawn through the middle of each piece. Tentacles regenerated, after three or four days, on the oral surface of each of the specimens.

A series of control experiments was made, in which the pieces after being fastened to the thread, were suspended in the water so

that the side of the individual would touch the side of the jar. By contracting the side of the column next the jar that edge of the foot was slowly brought over and after two days was attached to the glass. At the close of the next day, tentacles appeared at the oral end, which was still upright and diagonally opposite to the foot. Later, the anemone assumed a horizontal position.

Experiment XIII. As it seemed desirable to remove both contact and gravity as completely as possible, aboral pieces were placed in a tall jar, into which rapidly running water was brought by means of a glass tube. By adjusting the current of water, the pieces could be kept in motion for some time. The top of the jar was covered with muslin to prevent the escape of the anemones. Occasionally one of the pieces would become covered with air bubbles, rise to the surface and touch the muslin, or one might get out of the current of water and fall to the bottom. Such pieces were put back into motion as quickly as possible, always before they had become attached to the jar. While for these reasons, the experiment was not a complete success, yet the few pieces which had remained stationary were in contact with a surface for so short a time, it did not seem that the results would be effected. A few of the pieces regenerated tentacles in five days, and the others shortly afterwards, showing that tentacles can regenerate in pieces which are wholly unattached. The delay in the development may have been caused by the constant motion which seemed to prevent the pieces from expanding so that they remained in a more or less contracted state during the entire time of the experiment, or to the water being colder.

Experiment XIV. In the preceeding experiments, tentacles had regenerated at the oral end which was also the cut-surface. I tried to discover if it were possible to induce tentacles to grow from the side of the column at right angles to the axis of the individual by placing both the oral and aboral ends in contact with a solid body. A number of aboral pieces were placed vertically on a glass slide, with another slide supported by wooden pieces about a quarter of an inch above it. In this way, the oral surface was kept close against a solid structure, and while in this position no tentacles regenerated on the pieces. One of the smallest pieces, however, rolled over between the slides, and later a few tentacles regenerated at one end, undoubtedly the oral end. After about two weeks the other pieces died, and were thrown away. The question of contact may not have been the only one concerned here, as there

was enough pressure on the pieces to prevent their expansion in an oral-aboral direction.

Experiment XV. To remove the pressure and still keep the oral surface in contact with a solid structure, aboral pieces were tied to a glass slide with their oral surface pressing against the glass. This arrangement gave a possibility of expansion in a vertical direction with the pedal disk exposed. In spite of the fact that the pieces were tied as securely as possible, many of them pulled from under the thread at one side and rolled over. After several attempts, one piece of considerable size was fastened so securely to the slide that it did not free itself at once, and after three days two tentacles which had grown out from the oral disk pushed along the slide and appeared at the edge of the anemone. The next day, the piece drew out from under the string and turned over. Smaller pieces which were fastened to the slide, died without regenerating. Very short, aboral pieces placed with the pedal disk up, did not turn themselves over, but died after ten days or more without regenerating either tentacles or a new foot. If the pieces turned over, they would fasten by the old disk, and often regenerate tentacles at the oral surface.

The readjustment of pieces to the original orientation.

In my preliminary paper, I showed that some pieces had apparently a different orientation from that of the individuals from which they had been cut. This was shown in sections by finding horizontal mesenteries which were at right angles to the oesophagus and tentacles. There was evidence showing that after the piece had become attached, a rearrangment of the old mesenteries in a perpendicular direction had taken place. »This effort toward a readjustment is seen first in the region of the new oesophagus and later extends over the whole piece. . . . The way in which mesenteries which are fastened at either end to the body wall can move and adapt themselves to a new axis of the body is not clear at present, but the suggestion may be made that unequal growth in different regions might allow one end to push up and the other to settle down.«

By giving special attention to this point, I have now found in a large majority of those pieces which were cut from the aboral end (line *a—b*, Fig. 1) and in those specimens which had been divided longitudinally, that the exposed surfaces closed in an oral-aboral

direction, and that the tentacles developed on the oral surface; the foot on the aboral surface, and that a change in orientation occurred in only a small number of individuals and was for less frequent than at first supposed. The new tissue at the place of the cut is exceedingly sensitive and a slight disturbance causes it to contract. Thus, in preserved material the side containing the scar is apt to be the shortest and a slant is given to the mesenteries which the living

Fig. 4.

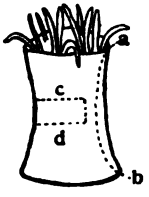


Fig. 5.

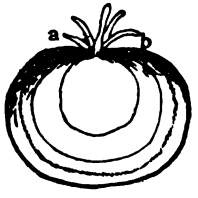


Fig. 6.

specimen did not have. There were, however, some very interesting exceptions shown by long, slender pieces cut from the column as shown by line *a*—*b*, Fig. 4. Such pieces, if very small, roll in along the exposed surface, so that the oral end (*a*) will come in contact with the aboral end (*b*) see Fig. 5. Figs. 5, 6, 7 and 8 show the development in one of the pieces. The presence of the vertical, orange bands were of so great importance in determining

Fig. 7.

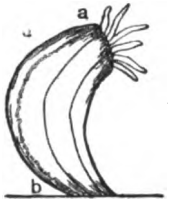
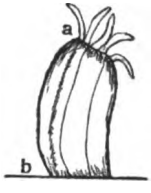


Fig. 8.



the orientation of these pieces, that any pieces lacking the bands were not considered. Fig. 5 was drawn ten days after the cut, and shows a few tentacles which have pushed out from the exposed side. It would be impossible, at this point, to determine whether the tentacles were on either the oral or aboral end, or on both. The drawing made two days later (Fig. 6) shows a growth, at one side of the tentacles, so that the aboral end (*b*) was slightly removed from the oral end (*a*). The day following, the piece was fastened at *b* and the column considerably bent (see Fig. 7). The straightening of the anemone followed (see Fig. 8); and in two or

three days it was nearly normal in position. The orientation of Fig. 5 would, at first, seem to be changed, but, by comparison with the later figures, it is evident that it was only an apparent change which was not permanent, and that the original orientation was quickly obtained by means of the rapid growth of new tissue in the region of the cut. Although there was no way of demonstrating that the tentacles developed on the oral rather than the aboral end; that is, that *a* in Fig. 4 corresponds to *a* in Fig. 6 rather than to *b* in this and in the following figures, yet in the light of the other experiments which were made at that time, it seems to me undoubtedly that this is the true interpretation.

Fig. 9. Fig. 10. Fig. 11.

Another example is shown by a specimen cut as indicated by *c—d* Fig. 4. The whole cut edge closed bringing the two ends and also the oral and aboral surfaces together as shown by a side view Fig. 9 and by an end view, Fig. 10. Both drawings which were made ten days after the operation show tentacles regenerating at the scar. Two days later, the aboral end had begun to push away (see Fig. 11), but the piece, which was very small, died before it had developed farther. Other specimens were under close observation, and they all showed that the oral-aboral axis, which at first appeared changed, straightened itself out. Even the smallest pieces capable of regeneration have a decided polarity.

In my previous paper I showed that the ocsophagus which regenerates in small aboral pieces is lined by endoderm. It may be well to state here that there is nothing in the more recent experiments which effect the conclusion then reached in regard to the regeneration of the oesophagus.

Conclusions.

1) Tentacles regenerate at the oral end of aboral pieces cut at different levels; even on very short pieces.

2) The absence of the pedal disk in a piece does not effect the regeneration of tentacles, and the time required for the formation of a new disk is longer than that for new tentacles.

3) A cut in the column or in the oral ring does not cause a growth of tentacles unless, in the latter case, some have been lost. If tentacles have been removed, new ones generally regenerate at the place of the cut, but they may be intercalated at intervals around the oral ring.

4) A pedal disk regenerates only at the aboral end of a piece, and tentacles only on the oral end of pieces (with the exception of a few heteromorphic tentacles on very small oral pieces).

5) Neither gravity nor contact effect the regeneration of tentacles, or the orientation of the individual.

6) The apparent change of orientation is only found in very small pieces, which, however, by means of rapid growth at the place of the cut ultimately assume the same orientation at that of the individuals from which they were cut, thus showing that even the smallest pieces have a definite polarity.

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Zusammenfassung.

1) Tentakel regeneriren sich am oralen Ende von aboralen Stücken, welche in verschiedener Höhe abgeschnitten wurden, auch an sehr kurzen Stücken.

2) Die Abwesenheit der Fußscheibe bei einem Stück hat auf die Regeneration der Tentakel keinen Einfluss. Auch ist die zur Regeneration einer neuen Fußscheibe erforderliche Zeit größer als die zur Tentakelregeneration nöthige.

3) Ein Schnitt durch die Columna oder den Mundring veranlasst kein Tentakelwachsthum, es sei denn, dass im letzteren Falle deren einige verloren gegangen sind. Sind Tentakel entfernt worden, so regeneriren sich im Allgemeinen deren neue an der Schnittstelle, sie können aber im Umkreise des Mundrings in Intervallen eingeschaltet werden.

4) Eine Fußscheibe regenerirt sich stets nur am aboralen, Tentakel nur am oralen Ende eines Stücks (mit Ausnahme einiger heteromorphischer Tentakel an ganz kleinen oralen Stücken).

5) Weder Schwerkraft noch Kontakt üben auf die Regeneration von Tentakeln oder auf die Orientirung der Individuen einen Einfluss aus.

6) Eine scheinbare Orientirungsänderung findet man lediglich bei sehr kleinen Stücken; auch diese jedoch nehmen mit Hilfe rapiden Wachsthums an der Schnittstelle schließlich dieselbe Orientirung an wie die Individuen, von denen sie abgetrennt wurden, und zeigen auf diese Weise, dass selbst den kleinsten Stücken eine ausgesprochene Polarität innewohnt.
